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Planctomycetes in Lakes: Poor or Strong Competitors for Phosphorus?

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Experiments were conducted with water samples from two perialpine lakes with differing eutrophication status in order to examine the effects of inorganic-nutrient amendments (nitrogen as NO_3^- or NH_4^+ and phosphorus as PO_4^{3-}) on the dynamics, structure, and composition of *Planctomycetes* and to test the hypothesis that the community structure of *Planctomycetes* members and that of the other bacteria (without *Planctomycetes*, here referred to as bacteria-wP, the most represented groups within the community) would be similarly impacted by nutrient additions. Initial samples were characterized by high total nitrogen-to-total phosphorus ratios (range, 39 to 55), suggesting P rather than N was the limiting nutrient for microbial communities. Consistent with this, P additions stimulated phytoplankton growth and affected the community structure of bacteria-wP but, surprisingly, not that of *Planctomycetes*. N additions did not significantly affect the community structures of bacteria-wP and *Planctomycetes* or the *Planctomycetes* phylotype composition. The estimated generation time of *Planctomycetes* was 123 h. These findings could suggest that the generally well-accepted statement that bacteria (as a whole) are superior to phytoplankton in the ability to obtain phosphorus under P limitation might actually not hold for *Planctomycetes*. *Planctomycetes* might be poor competitors for P that do not respond quickly to the nutrient supply, which may help explain why their abundance is low in aquatic systems. The alternative view that *Planctomycetes* could be strong competitors for P (storing it) is also discussed. Our findings highlight the need for further studies examining *Planctomycetes*-phosphorus relationships in aquatic ecosystems.

Planctomycetes are still among the less known bacterial phyla in terms of both dynamics and diversity, although the number of studies focusing on them has substantially increased during the past 2 decades (references 1 and 2 and references therein). In aquatic ecosystems, they represent between 0 and 11% of planktonic prokaryotic communities (3, 4, 5, 6, 7). Despite this low abundance, the role of *Planctomycetes* in aquatic ecosystem functioning is increasingly recognized, given their involvement in nitrogen and carbon cycling (6, 8, 9, 10). Field studies have shown increases in abundances and changes in the composition of *Planctomycetes* during or after phytoplankton or cyanobacterial blooms (7, 11). In line with this, studies using genomic approaches have shown that *Planctomycetes* possess genes involved in the degradation of plant detritus and algal polymers (12, 13).

While these new data support the idea that *Planctomycetes* play a major role in aquatic ecosystem functioning, little is known about factors controlling the dynamics of their members and why their abundance in the water columns of these ecosystems is so low. Understanding this requires examining responses and/or relationships of *Planctomycetes* to changes in environmental conditions (including resources such as inorganic nutrients) and/or the impacts of viruses and grazers, since the abundance of an organism at a given time is the outcome of the balance between growth and mortality. Such information is scarce for *Planctomycetes*, partly because most such studies on freshwater bacterial communities have used general bacterial primers for amplification of 16S rRNA genes; these primers generally amplify the most represented bacterial phyla (*Actinobacteria*, *Proteobacteria*, and *Bacteroidetes*) well, but not *Planctomycetes*. Available data suggest that *Planctomycetes* might actually be maintained at low abundance in aquatic systems by mortality induced by viruses (5) and heterotrophic nanoflagellates (HNF) (6). On the other hand, the very few studies that have examined *Planctomycetes* growth have reported generation times above 100 h (14), suggesting that *Planctomycetes* might be slow-growing bacteria. However, the fact that *Planctomycetes* abundances increase during or after phytoplankton blooms (which are known to release large amounts of carbon, nitrogen, and phosphorus) indicates that dissolved organic matter (DOM) and inorganic-nutrient availability are key factors enhancing *Planctomycetes* growth and suggests that their low abundance might also be an indication that they are poor competitors for some nutrients. An experimental study has also shown strong increases in the abundance of pelagic *Planctomycetes* (estimated by fluorescent *in situ* hybridization) following additions of inorganic nitrogen and phosphorus, alone or in combination, in the absence of grazers (6). In many cases in that study, the increase in *Planctomycetes* abundances was greater than (N addition) or similar to those of dominant groups, such as betaproteobacteria (6). The few studies reporting on factors that might control *Planctomycetes* members also suggest an important role for inorganic nutrients (1, 15). For instance, different vertical distributions of *Planctomycetes* genera were reported in the suboxic zone of the Black Sea in relation to nitrate versus ammonium concentrations (15). Recently, we have also found in two perialpine lakes that variations in the number of genetic sequences belonging to dominant *Planctomycetes* phylotypes were mainly explained by nitrate and the ratio of inorganic N to inorganic P (1). Although these empirical relationships suggest different responses of members of the *Planctomycetes* to nutrients, how the supply of inorganic nutrients

Received 21 August 2013 **Accepted** 5 November 2013
Published ahead of print 22 November 2013
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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.02824-13.

doi:10.1128/AEM.02824-13

(a common event in aquatic ecosystems) affects *Planctomycetes* members is virtually unknown.

The aim of this study was to experimentally examine the effects of inorganic-nutrient (nitrate, ammonium, and phosphate) amendments on the dynamics (changes in taxonomical structure and abundance) of pelagic *Planctomycetes*. In order to understand whether *Planctomycetes* and the other bacteria (the most represented phyla in the communities) are impacted in similar ways by resource supply in aquatic systems, we compared variations in the structure of the *Planctomycetes* community, as determined using specific primers, to those of the other bacteria (referred to here as bacteria-wP, i.e., the bacterial community without *Planctomycetes*) as determined using general primers known to fail to amplify or to poorly amplify *Planctomycetes* (see references below). Because we showed in our previous study that *Planctomycetes* communities are structured similarly to the entire bacterial community, i.e., characterized by the existence of a few dominant operational taxonomic units (OTUs) and numerous rare OTUs (1), we expected that inorganic-nutrient amendments could have similar impacts (significant changes or no change at all) on the community structures of *Planctomycetes* and bacteria-wP.

(This work was part of the fulfillment of the requirements for a Ph.D. [Doctorat d'Université] degree from Université de Grenoble by T. Pollet.)

MATERIALS AND METHODS

Experiment design. The samples used in the experiments in the present study were collected at 2-m depth in two French deep perialpine lakes with different eutrophication status: Lake Annecy (oligotrophic) and Lake Bourget (mesotrophic). Four microcosm experiments were conducted during the summer months in 2009 and 2010. For each experiment, water samples were prefiltered through a 200-μm net to eliminate metazooplankton. All incubations were performed *in situ* at the 2-m depth in 4-liter transparent polycarbonate bottles (Nalgene); they lasted 3 days for experiments with inorganic phosphorus or 4 days for experiments with inorganic nitrogen.

Three of the four experiments were conducted to evaluate the impacts of inorganic N additions on *Planctomycetes*. The first experiment (experiment 1) was performed with samples from Lake Bourget and started on 27 July 2009. The second (experiment 2) and third (experiment 3) experiments were performed with samples from Lake Annecy and started on 10 August and 24 August 2009, respectively. Treatments were either unamended (control, no nutrient added) or enriched with ammonium (NH_4^+ as ammonium chloride; Sigma) or nitrate (NO_3^- as calcium nitrate; Sigma), with each of these N sources added at a final concentration of 10 μM. Each treatment was prepared in four replicates.

The fourth experiment (experiment 4) was conducted to evaluate the impacts of inorganic phosphorus additions on *Planctomycetes* and started on 9 August 2010. This experiment was performed with samples from Lake Annecy, which is known to have very low P concentrations (total phosphorus, generally <8 μg liter^{-1} [16]). Treatments were either unamended (control, no nutrient solution added) or enriched with phosphate (PO_4^{3-} as K_3PO_4; Sigma) at final concentrations of either 0.21 μM or 0.64 μM. The treatment with P at 0.21 μM was prepared to provide approximately twice the highest phosphate concentration often found in August in the top 5 m in the lake (~0.12 μM), while the treatment with P at 0.64 μM was prepared to mimic mesotrophic conditions. Each treatment was prepared in triplicate.

For each of these experiments, subsamples were taken prior to nutrient additions, a few minutes after the additions (T_0; initial values), and then every 24 h (T_{24}, T_{48}, T_{72}, and T_{96}) for analyses of variables. Inorganic-nutrient (NO_3^-, NH_4^+, and PO_4^{3-}) concentrations were analyzed only in subsamples taken prior to the additions. Total bacterial abundances and the community structure of *Planctomycetes* and bacteria-wP were analyzed in subsamples taken every 24 h, from T_0 for all four experiments. For experiment 3, in addition to the variables mentioned above, we performed detailed analyses of *Planctomycetes* communities at a finer taxonomic resolution, i.e., at the OTU level, and estimated the abundances of *Planctomycetes* and HNF at T_0 and T_{48}. For experiment 4, because P concentrations are often very low in Lake Annecy (the samples used in this case), chlorophyll *a* (Chl *a*) concentrations were estimated at the beginning and at the end of incubation in order to check whether phytoplankton was P limited, as the activity of these autotrophs may affect bacterial community structure (through competition for the nutrient or release of DOM).

Nutrient and chlorophyll *a* concentrations. Inorganic-nutrient (nitrate, ammonium, and phosphate) concentrations were analyzed using standard colorimetric methods (Association Française de Normalisation [AFNOR] NF EN 1189, NF T90-015, and NF EN ISO 26777). Chl *a* concentrations were determined by spectrophotometry (17) after filtration of water samples on Whatman GF/F filters.

Total bacterial and *Planctomycetes* abundances. Total bacterial abundances were estimated from formaldehyde-preserved samples (2% final concentration) using a flow cytometer (FacsCalibur; Becton, Dickinson) equipped with a laser emitting at 488 nm. Yellow-green 0.92-μm fluorescent latex beads were used as an internal standard. Samples were run at low speed (10 to 12 μl min^{-1}). Prior to analyses, subsamples (500 μl) of the formaldehyde-preserved samples were spiked with 1 μl of the working solution (stock solution diluted 10 times) of the nucleic acid dye SYBR green II to stain bacteria. Analyses were performed after 15-min incubation of the prepared subsamples in the dark at room temperature.

To estimate *Planctomycetes* abundances, 10-ml water samples were fixed with paraformaldehyde (final concentration, 4%) overnight at 4°C and then filtered through 0.2-μm-pore-size polycarbonate membrane filters rinsed with MilliQ water. The abundances of *Planctomycetes* collected on these filters were estimated using the probe PLA886 (3) and the catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) technique (18). Briefly, the filters were first embedded in agarose (0.1% [wt/vol]). They were then incubated in a fresh lysozyme solution (10 mg ml^{-1}) at 37°C for 1 h for cell wall permeabilization and then washed with MilliQ water, dehydrated with 96% ethanol, and dried at room temperature. The filters were incubated in 0.01 M HCl solution at room temperature for 10 min to inhibit endogenous peroxidases. Each filter was cut into several small sections. Each section was then covered with 300 μl of hybridization buffer plus 1 μl of probe working solution (50 ng μl^{-1}) and incubated at 35°C for 2 h. After incubation, the filters were washed in a preheated washing buffer at 37°C for 10 min and then incubated again in 1× phosphate-buffered saline (PBS) for 15 min at room temperature. At the end of this incubation, the filters were covered with a solution containing 1,000 μl of the amplification buffer, 10 μl of an H_2O_2 solution (1 μl H_2O_2 plus 200 μl 10% SDS), and 2.5 μl of the fluorescently labeled tyramide and then incubated on a rotating shaker at 37°C for 15 min in the dark. The filters were then washed in 1× PBS for 10 min in the dark and then in MilliQ water and finally dried with absolute ethanol. Counts were performed under an epifluorescence microscope (Olympus BX-61) equipped with the appropriate light filters (for Cy3 and DAPI [4′,6-diamidino-2-phenylindole]). At least 300 stained cells were counted on a random basis for each part of the filter (three parts for each filter) at a magnification of ×100 using immersion oil.

HNF abundances. Subsamples for HNF analyses were preserved using glutaraldehyde (final concentration, 1%). HNF were counted under an epifluorescence microscope (Olympus BX-61) after staining preserved samples with DAPI (3-mg liter^{-1} final concentration) and filtration through 0.8-μm polycarbonate filters.

Bacterial and *Planctomycetes* community structures. Subsamples taken during experiments for characterization of bacterial and *Planctomycetes* community structures (350 ml) were first filtered through a 2-μm-pore-size polycarbonate membrane filter (Nuclepore) to eliminate larger

eukaryotes. The bacterioplankton remaining in the filtrate were then collected through gentle filtration (using a peristaltic pump) on 0.2-μm-pore-size polycarbonate filters (Nuclepore), which were stored at −20°C until the nucleic acid extraction was performed. Nucleic acid extraction was carried out as described previously (19).

PCR amplifications were performed with the PTC-100 Thermal Cycler (MJ Research Inc.). The PCR mixtures (50-μl volumes) contained approximately 30 ng of extracted DNA, 10× *Taq* reaction buffer (Eurobio), 120 μM each deoxynucleotide, 1 μM *Planctomycetes*-specific (PLA352F-GC/PLA920R [20, 21]) or bacterial (358F-GC/907RM [22, 23]) primers, bovine serum albumin (Sigma; 0.5-mg ml^{-1} final concentration), and 1.25 U *Taq* DNA polymerase (Eurobluetaq; Eurobio). The general primers 358F-GC/907RM were used to amplify sequences of the bacteria-wP, i.e., the bacterial community excluding *Planctomycetes*. In previous studies, including in Lake Annecy and Lake Bourget, this primer set was indeed found either not to detect *Planctomycetes* or to detect only a few sequences belonging to them, less than 1% of the total number of bacterial sequences (24, 25; R. D. Tadonléké, T. Pollet, P. van Rijswijk, B. Leberre, and J. J. Middelburg, unpublished data). After incubation at 96°C for 5 min, a touchdown PCR was performed using 10 cycles consisting of denaturation at 96°C for 1 min, annealing at 68°C (for *Planctomycetes*) or 65°C (for bacteria-wP) for 1 min, and primer extension at 72°C for 1 min. The annealing temperature was decreased by 1°C every cycle until the touchdown temperature of 58°C (for *Planctomycetes*) or 55°C (for bacteria-wP) was reached. Twenty (for the *Planctomycetes* PCR protocol) and 16 (for the bacteria-wP PCR protocol) additional standard cycles were carried out at an annealing temperature of 58°C (for *Planctomycetes*) or 55°C (for bacteria-wP), and a final extension step was performed at 72°C for 5 min. The presence of PCR products was checked by analyzing 7 μl of products on 1% agarose gels.

The denaturing gradient gel electrophoresis (DGGE) technique was used to characterize the community structure of *Planctomycetes* and that of bacteria-wP. Since this technique is unlikely to detect OTUs accounting for less than 1% of the total community (22), the bacterial community detected with the general primers in this study was considered free of *Planctomycetes* and is therefore called bacteria-wP. DGGE of the PCR products was performed on a 6% (wt/vol) polyacrylamide gel with a linear gradient of urea and formamide increasing from 50% to 70% from the top to the bottom of the gel for the *Planctomycetes* PCR products and from 40% to 80% for the bacteria-wP PCR products. Electrophoresis was performed in 1× Tris-acetate-EDTA (TAE) buffer at 60°C at a constant voltage of 120 V for 18 h. Gels were then stained for 1 h in 1× TAE buffer containing SYBR gold (1:5,000 final concentration), rinsed with distilled water, and visualized and enumerated on a UV transilluminator (Tex-35M; Bioblock Scientific). The gel pictures were analyzed using Gel Compare II software, and the Shannon diversity index (H') was estimated for each profile using the software Past (http://folk.uio.no/ohammer/past). As mentioned above, the responses of *Planctomycetes* in terms of variations in the community structure were compared to those of bacteria-wP in order to understand whether *Planctomycetes* and the dominant bacteria are impacted in the same way by the nutrient supply.

The detailed analysis of *Planctomycetes* OTU composition in experiment 3 was carried out using a cloning-sequencing approach. The PCR products were cloned using the TOPO TA cloning kit (Invitrogen) according to the manufacturer's instructions. For each sample, 96 positive clones (white colonies) were randomly selected, verified by PCR using the commercial primer M13 (Promega), and finally sequenced (GATC Biotech). The sequences were then edited, aligned using Genedoc (26), and checked for chimeras using Bellerophon (27) and Check Chimera in the Ribosomal Database Project (RDP) (28). OTUs were defined on the basis of ≥98% sequence identity, and the diversity was estimated by the Shannon diversity index. The Chao1 and abundance-based coverage estimators of species richness were calculated using the software EstimateS (http://viceroy.eeb.uconn.edu/estimates). Rarefaction curves were also calculated, using the software PAST (http://folk.uio.no/ohammer/past). A phylogenetic analysis was performed by neighbor joining using MEGA4 software (http://www.megasoftware.net) to determine the affiliation of each OTU to the different *Planctomycetes* clusters currently known.

Statistical and DGGE analyses. Changes in bacterial and *Planctomycetes* abundances, HNF abundance (experiment 3), and Chl *a* concentrations (experiment 4) were evaluated using a two-way (nutrient × time) analysis of variance (ANOVA). DGGE gel banding patterns were analyzed using Gel-Compare II. Each band was described by its position and its relative intensity in the profiles. For each profile, the Shannon diversity index was estimated. Comparative analysis of DGGE fingerprints was carried out with PAST and Primer 6 software. Ordination of Bray-Curtis similarities among normalized sample profiles was performed by nonmetric multidimensional scaling (MDS). This ordination technique was used to determine relationships among sample profiles, considered representative of bacterial or *Planctomycetes* community structures in each sample. The different groups (ellipses) presented in MDS were statistically defined according to the Bray-Curtis index using Primer 6 software. The degree to which the plot matches the similarity matrix can be judged by examining the stress values, defined here as Kruskal's stress formula (29). Stress values lower than 0.1 indicate good ordination, i.e., with few risks of misinterpretation of patterns (30). To test the null hypothesis that there was no significant difference between the groups discriminated according to the MDS analysis, we conducted an analysis of similarities with the subroutine ANOSIM of the software Primer. ANOSIM is a nonparametric test designed to perform statistical comparisons of multivariate data sets in a manner similar to univariate techniques (ANOVA) (31). ANOSIM first calculates the R statistic, which displays the degree of separation between groups. Complete separation is indicated by an R value of 1, and an R value of 0 suggests no separation. Having determined R, ANOSIM assigns samples randomly to different groups to generate a null distribution of R (Monte Carlo test) in order to test whether within-group samples are more closely related to each other than would be expected by chance. UNIFRAC analysis (32) was used to statistically compare the different clone libraries.

Nucleotide sequence accession numbers. Novel sequences determined in this study were deposited in GenBank under accession numbers KF704718 to KF704741.

RESULTS

Initial nutrient concentrations. The nutrient data clearly indicated that initial samples (i.e., prior to nutrient additions) used in our experiments were relatively rich in nitrogen but very poor in phosphorus (Table 1). As a consequence, the ratio of dissolved inorganic nitrogen (DIN) to dissolved inorganic phosphorus (DIP) and that of total nitrogen (TN) to total phosphorus (TP) were >36, ranging from 37 to 130 and from 39 to 55, respectively (Table 1).

Impacts of nutrient additions on total bacterial abundances, *Planctomycetes* abundances, HNF abundances, and Chl *a* concentrations. During the four experiments, nutrient (N or P) additions generally had no significant effects on total bacterial abundances. Total bacterial abundances varied significantly only with time (two-way ANOVA; $P < 0.05$) (Fig. 1A to D).

Variations in *Planctomycetes* abundances during experiment 3 (samples from Lake Annecy) are presented in Fig. 2. Time, treatment, and their interactions (time × treatment) had significant effects on these abundances ($P < 0.001$). However, the effects of treatment occurred only at T_{48}, when *Planctomycetes* abundances were significantly lower in treatment with ammonium or nitrate than in the control (Fig. 2A). Except in the control, *Planctomycetes* abundances and contribution to total prokaryote abundance decreased from T_0 to T_{48} (Fig. 2A and B), in contrast to total bacterial abundance, which did not decrease at all over time (Fig. 1C). Vari-

TABLE 1 Nutrient concentrations measured at the beginning of each experiment prior to nutrient addition

Parameter[a] (units)	Value			
	Nitrogen addition expt			
	Bourget expt 1 (27 July 2009)	Annecy expt 2 (10 August 2009)	Annecy expt 3 (24 August 2009)	Phosphate addition expt (Annecy expt 4; 9 August 2010)
NH_4^+ (mg liter^{-1})	0.009	0.009	0.01	0.002
NO_3^- (mg liter^{-1})	0.14	0.12	0.12	0.11
TN (mg liter^{-1})	0.31	0.22	0.22	0.27
PO_4^{3-} (mg liter^{-1})	0.004	0.001	0.001	0.003
TP (mg liter^{-1})	0.008	0.004	0.004	0.005
DIN:DIP	37.3	129	130	37.3
TN:TP	38.8	55	55	54

[a] TN, total nitrogen; NH_4^+, ammonium; NO_3^-, nitrates; TP, total phosphorus; PO_4^{3-}, phosphates; DIP, dissolved inorganic phosphorus; DIN, dissolved inorganic nitrogen; DIN:DIP and TN:TP, nitrogen-to-phosphorus ratios.

ations in the *Planctomycetes* abundances in the control (the only sample with a general increasing trend in abundance) yielded a growth rate estimate of 0.135 day^{-1}, assuming an exponential model. This corresponded to a generation time of ~123 h, i.e., more than 5 days.

Time, nutrients, and their interactions (time × nutrients) also had significant effects on HNF abundances in experiment 3 ($P < 0.01$). Like *Planctomycetes* abundances, HNF abundances decreased from T_0 to T_{48} and were lower in nitrogen-amended treatments than in the control at T_{48} (Fig. 2C). Figure S1 in the supplemental material shows variations in Chl *a* concentrations measured in the experiment with phosphorus additions (experiment 4). At T_0, the Chl *a* concentration was 1.07 mg m^{-3}. Concentrations at T_{72} in the control were very similar to those at T_0 and significantly lower than those in treatments with phosphorus at T_{72} (1.68 and 1.94 mg m^{-3} for treatments P1 and P2, respectively; $P < 0.01$), suggesting that incubation alone and potential bottle effects did not significantly affect the communities under study and indicating P stimulation of phytoplankton (Table 1).

Impacts of nutrient additions on the community structure of bacteria-wP and *Planctomycetes*. For each experiment, the DGGE banding patterns of the different replicates of each treatment were similar at each sampling time point. The DGGE profiles used for nonmetric MDS analyses were obtained after pooling aliquots of DNA extracts from different replicates of each treatment at each sampling time point, since all the replicates from an experiment (48 or 60) could not be placed in the same gel. As might be expected, the number of DGGE bands obtained for bacteria-wP (ranges, 16 to 20, 26 to 27, 21 to 23, and 20 to 29 for experiments 1, 2, 3, and 4, respectively) was always much higher

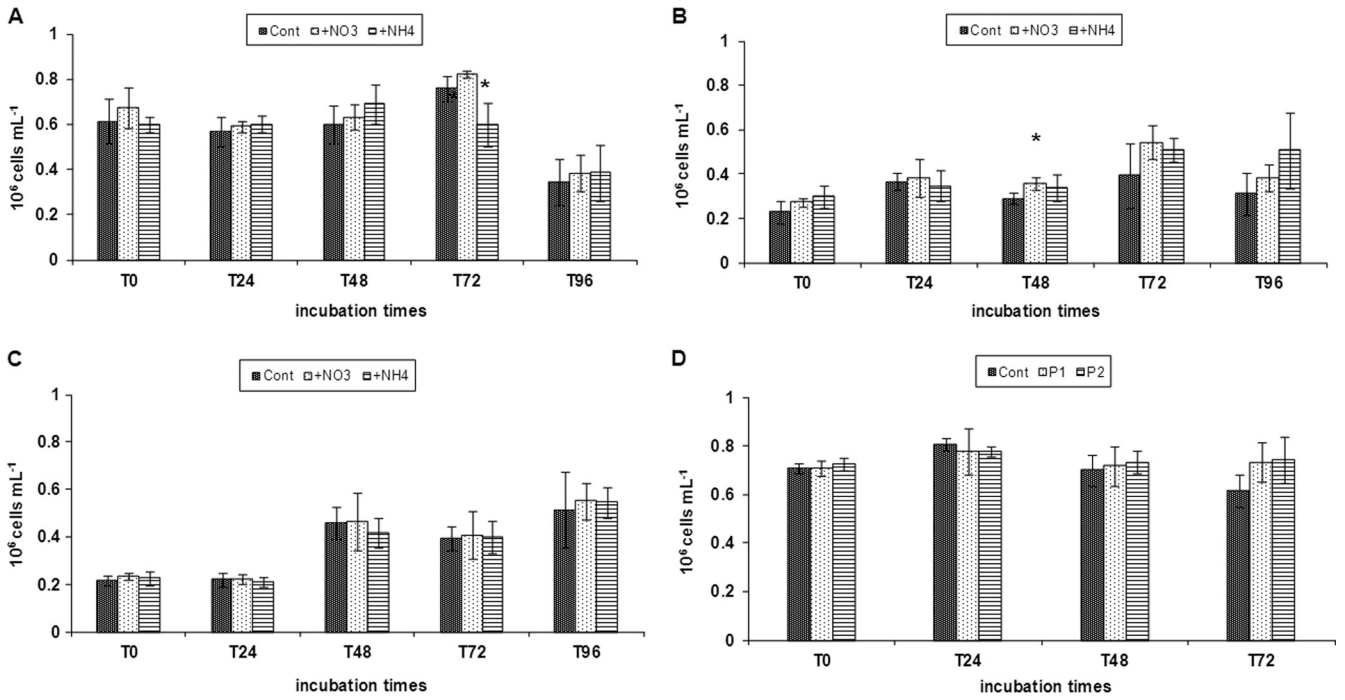

FIG 1 Variations in the total bacterial abundances after addition of NO_3^- or NH_4^+ (experiment 1, in Lake Bourget [A], and experiments 2 and 3, in Lake Annecy [B and C]) or phosphate (experiment 4, in Lake Annecy [D]). The asterisks indicate the sampling time points for which treatments were significantly different from the control. The error bars indicate standard deviations.

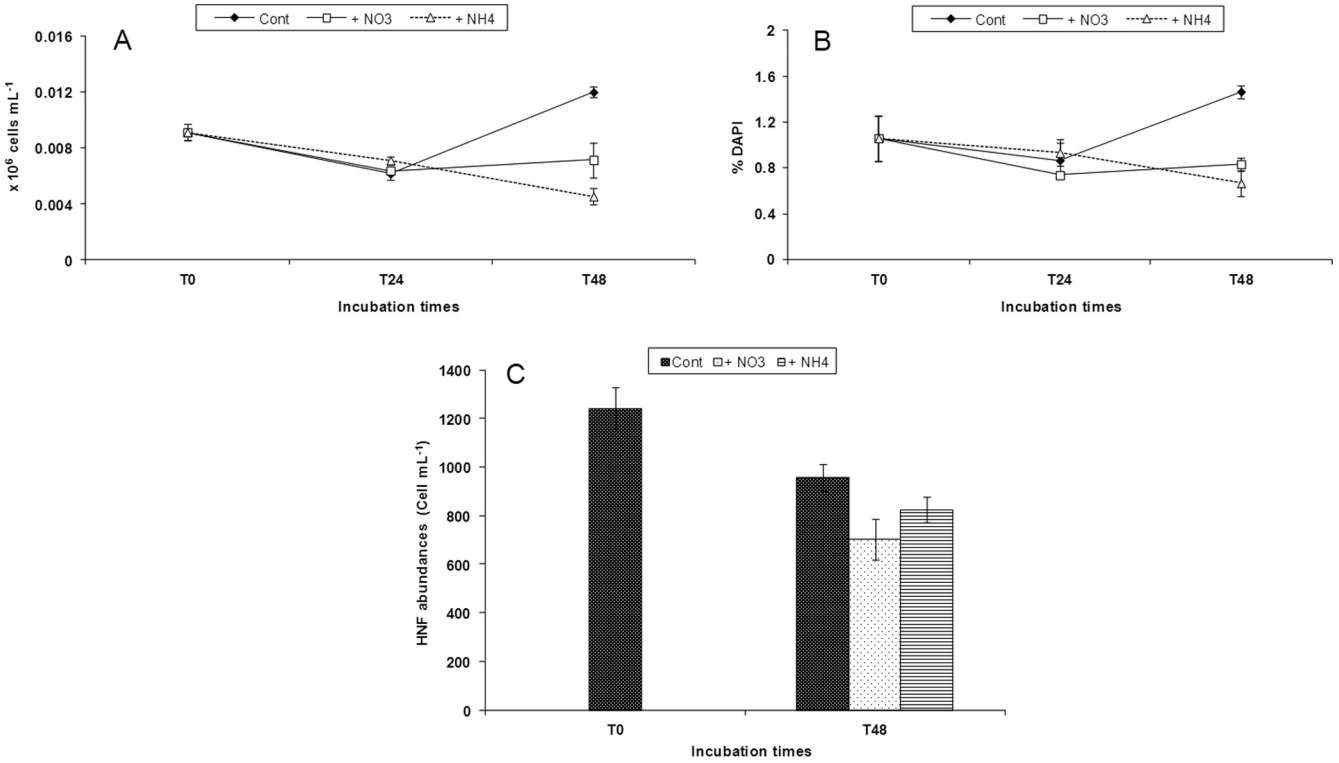

FIG 2 Variations in *Planctomycetes* abundances (A), in their relative contribution to the total number of prokaryotic cells estimated with DAPI (B), and in HNF abundances (C) during experiment 3 (Lake Annecy). The error bars indicate standard deviations.

than that of *Planctomycetes* (ranges, 8 to 10, 7 to 8, 11 to 14, and 7 to 10 for experiments 1, 2, 3, and 4, respectively). For each time point, no significant difference was found between the treatments and the control in the Shannon diversity index estimated for bacteria-wP or for *Planctomycetes* communities based on the number and intensity of bands (data not shown).

MDS ordination plots obtained from the different DGGE gels are presented in Fig. 3. As indicated by the goodness of fit of the Kruskal stress values, which were always lower than 0.1, the distances in the different MDS ordinations reflect relatively well the magnitude of similarity in DGGE patterns between samples. For each analysis, the nonparametric ANOSIM subroutine of Primer confirmed the robustness of the separation of the groups that were discriminated groups, as the *R* value was equal to 0.73 in one case and close to 1 in the other cases (Fig. 3A to H).

The plots showed that for experiments dealing with N additions (Fig. 3A to F), there were distinct community structures between the period T_0 to T_{48} and the period after T_{48} for both *Planctomycetes* and bacteria-wP for two experiments (1 and 2) out of three. For these N experiments, treatments (NH_4^+ and NO_3^-) and controls for each sampling time point are within the same ellipse (i.e., belong to the same group) in the MDS plots for both bacteria-wP (except for T_{48} in experiment 3) and *Planctomycetes* (Fig. 3A to F), which indicates a general lack of significant effects of N additions on the community structures of these bacteria. In contrast, as indicated by the fact that treatments (P1, 0.21 μM, or P2, 0.64 μM) and controls were no longer in the same ellipse after T_{24}, P additions (experiment 4) resulted in significant changes in the community structure of bacteria-wP compared to the controls after 24 h of incubation, even though no significant effects of P concentration were found (Fig. 3G). Interestingly, P amendments had, in contrast, no significant effects on *Planctomycetes* community structure, as for each sampling time point, treatments (P1 or P2) and controls were always in the same ellipse (Fig. 3H). Moreover, the *Planctomycetes* community structures were similar between sampling time points, in contrast to the bacterial-wP community structure, which showed a marked shift from the period T_0 to T_{48} to the period after T_{48} (Fig. 3G and H), as was observed for N additions.

Impacts of N additions on *Planctomycetes* composition. As mentioned above, we performed detailed analyses of *Planctomycetes* composition at T_0 and T_{48} for experiment 3. The four clone libraries obtained (T_0, T_{48}-control, T_{48}-NH_4, and T_{48}-NO_3) yielded 25 OTUs (accession numbers, KF704718 to KF704741). The values of the Chao1 estimator (8 ± 2.2, 13 ± 2.9, 28 ± 13.2, and 17 ± 5.5 for T_0, T_{48}-control, T_{48}-NO_3, and T_{48}-NH_4, respectively) and rarefaction curves (data not shown) indicated that we did not obtain a sufficient number of sequences to detect the whole *Planctomycetes* OTU richness in the different treatments. Among the 25 OTUs, 21 accounted for less than 5% of the sequences. Four (OTU1, OTU2, OTU3, and OTU4) were represented by a minimum of 5 sequences (≥5%) in at least one sample, and all four together accounted for 82.9% to 94.4% of the total *Planctomycetes* sequences (Fig. 4). OTU1, OTU2, and OTU3 belong to the genus *Pirellula/Blastopirellula*, while OTU4 was affiliated with the genus *Gemmata/Zavarzinella*. OTU1 was dominant in all four clone libraries, accounting for 38.7% to 55.4% of the sequences (Fig. 4). UNIFRAC analyses showed no significant differences between the three different clone libraries (T_{48}-control, T_{48}-NO_3, and T_{48}-NH_4) after 48 h of incubation, although some

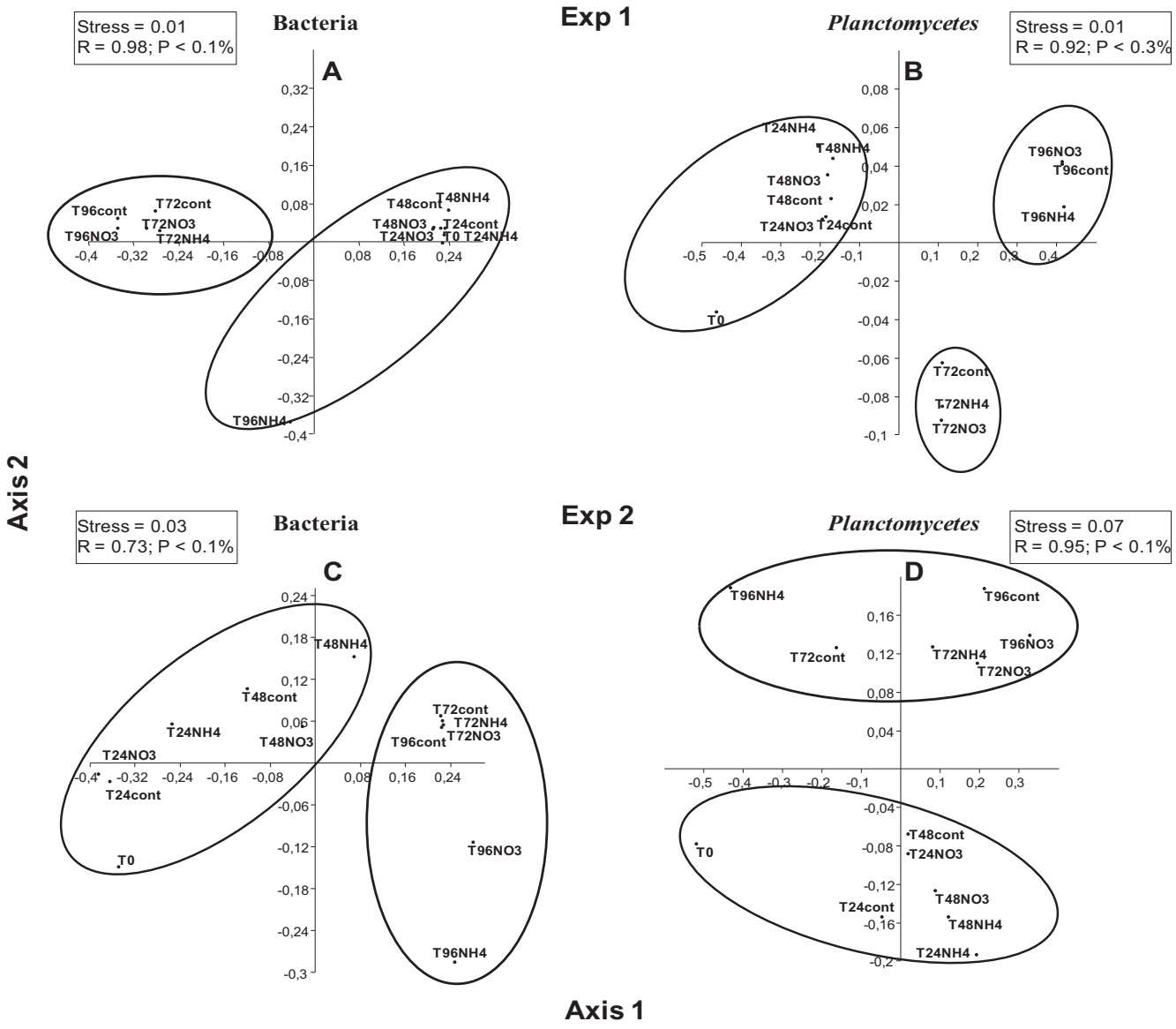

FIG 3 (A to D) Nonmetric MDS plots based on analysis of the different PCR-DGGE profiles obtained for *Planctomycetes* and bacteria-wP during experiment 1 in Lake Bourget (Exp 1) (A and B) and experiment 2 in Lake Annecy (Exp 2) (C and D). (E to H) Nonmetric MDS plots based on analysis of the different PCR-DGGE profiles obtained for *Planctomycetes* and bacteria-wP during experiment 3 in Lake Annecy (Exp 3) (E and F) and during experiment 4 (Exp 4) (G and H). The different groups (surrounded by ellipses) presented in MDS plots were discriminated according to the Bray-Curtis similarity. Stress, Kruskal's stress; *R* and *P* values, ANOSIM parameters. See Materials and Methods for details.

variations in the proportions of the dominant OTUs were observed ($P > 0.1$) (Fig. 4).

DISCUSSION

Understanding the impacts of inorganic nutrients (NO_3^-, NH_4^+, and PO_4^{3-}) on bacterioplankton is of great interest, since aquatic systems frequently receive inputs and pulses of these nutrients, which may accelerate their eutrophication. The effects of nutrient additions on bacterial communities and on dominant bacterial phyla have been studied extensively and are now well known (33, 34, 35, 36, 37, 38). However, while empirical studies suggest different responses of members of *Planctomycetes* to nutrients (1, 15), the impacts of the inorganic-nutrient supply on members of this nondominant but ecologically and functionally important bacterial phylum remain unknown. Because we showed in our previous study (1) that *Planctomycetes* communities are structured similarly to the entire bacterial community, i.e., characterized by the existence of few dominant OTUs and numerous rare OTUs, we predicted that inorganic-nutrient amendments could have similar impacts on the two communities.

Consistent with our previous findings during studies conducted in Lake Annecy and Lake Bourget in 2008 (1), the present study showed that *Planctomycetes* communities comprised a few (3) dominant OTUs and numerous (21) OTUs present in very low proportions (generally <2%) (Fig. 4). This strengthens our previous conclusion that *Planctomycetes* communities are structured

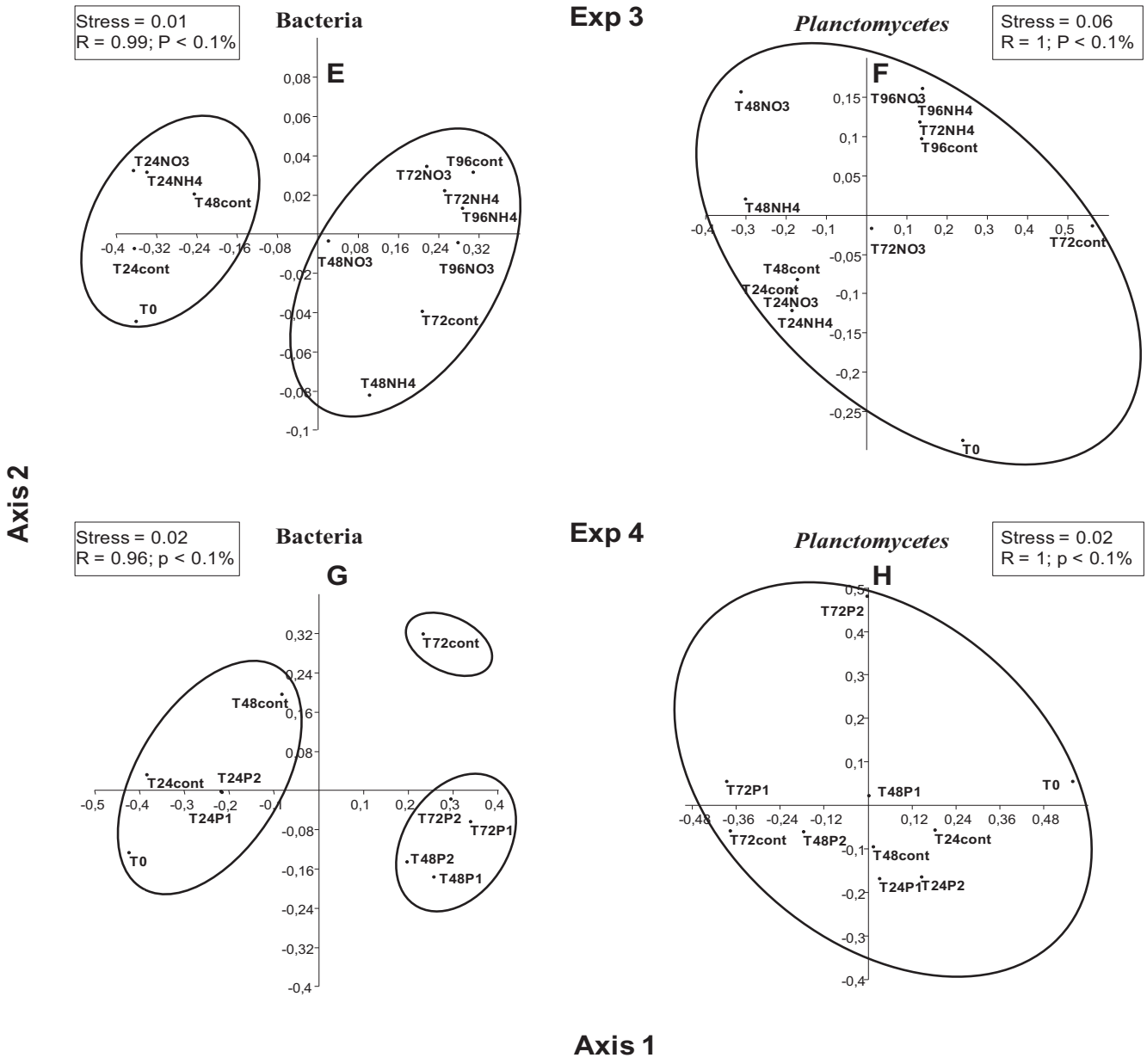

FIG 3 continued

similarly to the entire bacterial community (1). However, contrary to what we expected, P additions in this study significantly changed the community structure of bacteria-wP (i.e., the most represented bacterial groups) but not that of *Planctomycetes* (Fig. 3G to H). Moreover, the community structure of *Planctomycetes* was extremely stable throughout this experiment (similar from one sampling time point to another), while that of bacteria-wP exhibited a marked shift after T_{48} (Fig. 3G to H). Microbial communities in the experiment were undoubtedly limited by P prior to its supply, as indicated by stimulation of phytoplankton growth after P additions (see Fig. S1 in the supplemental material). This response was consistent with the very small P amounts in initial samples and with the TN:TP ratio being largely higher than 16 (by mass), the threshold beyond which P limitation of phytoplankton often occurs (39, 40). Bacterioplankton-phytoplankton interactions are very complex, and understanding them requires sophisticated approaches (41). We do not pretend that we have investigated or solved this issue here. Potential indirect influences of nutrient addition on bacterial community composition via stimulated growth of phytoplankton cannot be ruled out in this study. However, our findings and data from literature suggest that the responses of bacteria-wP to P additions were mainly direct effects of P (i.e., not mainly due to indirect effects of exudates released by the stimulated phytoplankton). First, if these responses were mainly due to exudates, *Planctomycetes* would have likely also responded, since they are known to increase during or after phytoplankton blooms in nature (7, 11). Second, experimental studies using grazer- and phytoplankton-free samples (i.e., filtered on

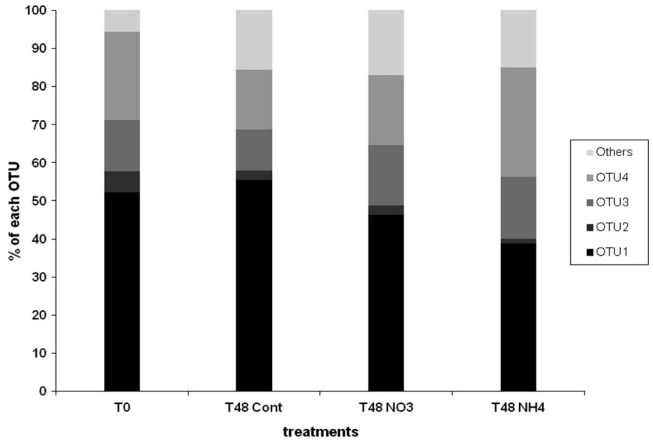

FIG 4 Changes in the relative abundances (percentages) of *Planctomycetes* OTUs found for each treatment at T_0 and T_{48} during experiment 3 performed in Lake Annecy.

0.8-μm filters) have shown P stimulation of bacterioplankton as a whole in Lake Annecy (42). Third, another study in the lake has shown on an annual scale that inorganic nutrients outweighed other physicochemical parameters (including Chl *a*) in explaining variance (>60% versus 12%) of bacterial community structure (as characterized with the same primers used here for bacteria-wP) (43). Finally, there is evidence indicating that under P limitation, bacterioplankton do not efficiently use phytoplankton exudates (44).

Bacterial communities are reported to be stronger competitors for P than phytoplankton, owing to their higher P uptake kinetics (45). The lack of responses of *Planctomycetes* to P additions, therefore, contrasted with this generally well-accepted knowledge based on studies considering bacteria as a whole (45). Although PCR-DGGE generally does not detect OTUs representing <1% of the total community and may overlook some changes in bacterial composition at a fine phylogenetic level (22), the technique has been shown to detect changes concerning dominant phylotypes within *Planctomycetes* targeted using specific primers (1). Hence, changes (if any) would have been detected by DGGE in the *Planctomycetes* community structure in this study following P addition if they were major. Our results therefore raise hypotheses about the ability of *Planctomycetes* to acquire P in natural environments within mixed microbial communities. One of the most consistent arguments is that *Planctomycetes* could be poor competitors for P acquisition compared to other bacteria and phytoplankton. Indeed, bacteria with high growth rates generally respond quickly to environmental changes, such as nutrient inputs, compared to bacteria with low growth rates (37). This view of *Planctomycetes* as slow-growing bacteria (as we hypothesized) is supported by the long generation time (slightly more than 5 days) found during this study, similar to that (~5 days) reported by the few studies that we are aware of on this aspect of *Planctomycetes* ecology (14). The possibility that grazing and viral lysis also influenced *Planctomycetes* abundance (5, 6) and therefore the estimated generation time cannot be excluded. Unfortunately, we do not have data on viruses to explore this view. However, we are not aware of any study reporting viruses as the main drivers of *Planctomycetes* abundance. Regarding grazing, if *Planctomycetes* were strongly controlled by HNF (e.g., eaten as fast as they were growing), the significant decrease in HNF abundance observed, for example, at T_{48} in the treatment with NO_3^- compared to the controls (Fig. 2C), would have likely resulted in increases in *Planctomycetes* abundance compared to the controls. This was not the case, supporting the idea of slow growth for *Planctomycetes*. Our results seem to be consistent with those of a recent study showing that natural bacteria may be extremely poor in P (46), an indication that they are not always as strong competitors for P as they are usually said to be, contradicting the generally admitted idea that bacteria are always nutrient-rich components of the plankton (46). Our findings could suggest that the generally admitted statement that bacteria are superior to phytoplankton in the ability to obtain phosphorus under P limitation might actually not hold for all bacterial phyla, and in particular for *Planctomycetes*. From this perspective, competition for P acquisition might be among the reasons why *Planctomycetes* are in low abundance in aquatic systems, although the lack of some specific resources not examined here may also be involved. However, although not supported by the low growth rate estimated here (0.135 day^{-1}) and in other studies (14), an alternative argument is that *Planctomycetes* could be strong competitors for P and therefore not P limited. Another argument is that *Planctomycetes* might have high affinity for phosphate, which results in little to no effects of additions of extra P. Unfortunately, data about *Planctomycetes* affinity for P are scarce, which limits comparisons. Similar to our results, a study in the oligomesotrophic Lake Piburger See (P concentration, ~15 μg $liter^{-1}$) has shown that *Actinobacteria* did not respond to a surplus of phosphorus, whereas other bacterial groups did (37). However, *Actinobacteria* are known to be able to accumulate P as polyphosphate within the cell (47), a capacity that may allow survival under P starvation and may help explain a lack of response to P addition. However, such a capacity, to our knowledge, has not yet been shown for *Planctomycetes*. In a study investigating the effects of oxygen deprivation on bacterioplankton, we found that *Planctomycetes* members were among the dominant bacterial phylotypes under oxygen-deficient conditions where bacterial cells were accumulating P (Tadonléké et al., unpublished). While this was suggestive of a relationship with P, it was not in oxygenated waters, as in this study, and remains to be demonstrated. Therefore, the observed lack of response of *Planctomycetes* to P addition highlights the need for further studies on *Planctomycetes*-phosphorus relationships to confirm whether they are strong or poor competitors for P.

Recent studies have suggested that inorganic-nitrogen sources are important nutrients in the dynamics and composition of *Planctomycetes* communities (1, 6, 15). Interestingly, the results from DGGE and clone libraries were similar, and both showed no significant effects of N addition on the global community structure of *Planctomycetes* or of the other bacteria. Moreover, while *Planctomycetes* abundances and contributions to total prokaryotic abundances in controls in our experiment 3 (e.g., from 0.87% to 1.47% [Fig. 2A and B]) slightly increased over time and were within the range of values generally reported in aquatic ecosystems (3, 4, 5, 6, 7), those in N-amended samples decreased over time and were lower than in controls (Fig. 2A and B). It should be noted, however, that the abundance of *Planctomycetes* might have been underestimated in this study, as the probe PLA886 does not detect all *Planctomycetes* (3). The lack of effects of nutrient supply on the structure of bacterial communities is common in environments where the added nutrient is already abundant in initial

samples, as was the case here for nitrogen. The TN:TP ratios in all our initial samples were indeed much higher than the Redfield ratio (N:P = 16), suggesting that N was not limiting for microbial communities (Table 1). However, even though N was not limiting, the temporal changes in the community structure of *Planctomycetes* were different from those of the bacteria-wP (the most represented bacterial groups) in two N experiments (experiment 1 and experiment 3) out of three (Fig. 3). In these two experiments, the temporal dynamics displayed two phases, with a shift at T_{48}, for bacteria-wP, whereas for *Planctomycetes*, it showed either no changes or three phases (Fig. 3A, B, E, and F). This supports the idea that the dynamics of *Planctomycetes* were different from those of the other members of the bacterioplankton.

High-throughput next-generation sequencing, such as pyrosequencing, would have likely provided more information on bacterial diversity and richness here. Unfortunately, at the time this study was performed, pyrosequencing was still very expensive. Given this cost, the number of samples to be analyzed (104), and the fact that DGGE and cloning-sequencing are relevant to the objectives of this study (which were not to investigate diversity), we concluded at that time that the cost/benefit ratio of using pyrosequencing was too high.

The reasons why the total abundance of *Planctomycetes* decreased in N-amended samples compared to the control (Fig. 2A and B) remain unclear to us, in particular since a similar trend was observed for HNF abundance, but not for total abundance of prokaryotes (Fig. 1 and 2). It is difficult to say whether this was due to selective removal of *Planctomycetes* by grazers (mixotrophic flagellates and HNF have been found to be important grazers of bacteria in the lake [48]) or to direct negative effects of N on some microbes (as N was already abundant in the milieu, additional N might have caused its concentration to become too high and therefore toxic to *Planctomycetes* that prefer moderate N concentrations). For whatever reason, these results seemed to contrast with those of another study reporting increases in *Planctomycetes* abundance following N additions in samples from humic freshwater ecosystems (6). Methodological differences could be among the reasons for these different results. For instance, the experiments (6) dealt with the dilution-regrowth approach and grazer-free samples. *Planctomycetes* were constrained by grazers in that study (6). In such situations, removal of grazers coupled with nutrient additions often result in positive responses of bacteria.

In conclusion, besides strengthening our previous conclusion that *Planctomycetes* communities in aquatic ecosystems are composed of a few dominant OTUs and numerous rare OTUs, this study showed that this bacterial phylum did not respond to P additions, while the other (dominant) bacteria and phytoplankton were significantly affected. The lack of significant effects of N additions on all the studied bacterial communities confirmed results commonly observed when the added nutrient is already abundant in the milieu. Our findings suggest that the generally well-accepted statement that bacteria are superior to phytoplankton in the ability to obtain phosphorus under P limitation might actually not hold for *Planctomycetes* and that members of the group could be poor competitors for P. Although not really supported by the long generation time found here for *Planctomycetes*, an alternative view could be that members of the bacterial phylum might be strong competitors for P, with the capacity to store it and therefore not to respond to its supply. Clearly, our findings highlight the need for further studies on *Planctomycetes*-phosphorus relationships in aquatic ecosystems. These will likely provide insight into their occurrence and temporal dynamics in aquatic ecosystems, in particular relative to other bacteria and to phytoplankton.

ACKNOWLEDGMENTS

T.P. was supported by a French Ministry of Research doctoral grant to R.D.T. The study was also supported by funding from the Cluster Environnement of the Rhône-Alpes Region (France) to R.D.T. T.P. received additional financial support from the Département Ecologie des Fôrets, des Prairies et des Milieux Aquatiques (EFPA) of INRA.

We are grateful to Pascal Perney, Laurent Espinat, Jean-Christophe Hustache, Pascal Chifflet, Aurélie Hebert, Danielle Lacroix, and Jérôme Lazzarotto, who provided help during the experiments and performed nutrient analyses. We thank the journal reviewers for their constructive comments.

REFERENCES

1. **Pollet T, Tadonléké DR, Humbert JF.** 2011. Spatio-temporal changes in the structure and composition of a less abundant bacterial phylum (*Planctomycetes*) in two peri-alpine lakes. Appl. Environ. Microbiol. **77:**4811–4821. http://dx.doi.org/10.1128/AEM.02697-10.
2. **Fuerst JA, Sagulenko E.** 2011. Beyond the bacterium: planctomycetes challenge our concepts of microbial structure and function. Nat. Rev. Microbiol. **9:**403–413. http://dx.doi.org/10.1038/nrmicro2578.
3. **Neef A, Amann R, Schlesner H, Schleifer KH.** 1998. Monitoring a widespread bacterial group: in situ detection of planctomycetes with 16S rRNA-targeted probes. Microbiology **144:**3257–3266. http://dx.doi.org/10.1099/00221287-144-12-3257.
4. **Gade D, Schlesner H, Glöckner FO, Amann R, Pfeiffer F, Thomm M.** 2004. Identification of planctomycetes with order-, genus-, and strain-specific 16S rRNA-targeted probes. Microb. Ecol. **47:**243–251. http://dx.doi.org/10.1007/s00248-003-1016-9.
5. **Bouvier T, del Giorgio PA.** 2007. Key role of selective viral-induced mortality in determining marine bacterial community composition. Environ. Microbiol. **9:**287–297. http://dx.doi.org/10.1111/j.1462-2920.2006.01137.x.
6. **Tadonléké RD.** 2007. Strong coupling between natural *Planctomycetes* and changes in the quality of dissolved organic matter in freshwater samples. FEMS Microbiol. Ecol. **59:**543–555. http://dx.doi.org/10.1111/j.1574-6941.2006.00222.x.
7. **Pizzetti I, Fuchs BM, Gerdts G, Wichels A, Wiltshire KH, Amann R.** 2011. Temporal variability of coastal *Planctomycetes* clades at Kabeltonne Station, North Sea. Appl. Environ. Microbiol. **77:**5009–5017. http://dx.doi.org/10.1128/AEM.02931-10.
8. **Strous M, Fuerst JA, Kramer EHM, Logemann S, Muyzer G, van de Pas-Schoonen KT, Webb R, Kuenen JG, Jetten MSM.** 1999. Missing lithotroph identified as new *Planctomycetes*. Nature **400:**446–449. http://dx.doi.org/10.1038/22749.
9. **Kuypers MMM, Sliekers AO, Lavik G, Schmid M, Jørgensen BB, Kuenen JG, Sinninghe Damsté JS, Strous M, Jetten MSM.** 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. Nature **422:**608–611. http://dx.doi.org/10.1038/nature01472.
10. **Schubert CJ, Durisch-Kaiser E, Wehrli B, Thamdrup B, Lam P, Kuypers MMM.** 2006. Anaerobic ammonium oxidation in a tropical freshwater system (Lake Tanganyika). Environ. Microbiol. **8:**1857–1863. http://dx.doi.org/10.1111/j.1462-2920.2006.01074.x.
11. **Morris RM, Longnecker K, Giovannoni SJ.** 2006. Pirellula and OM43 are among the dominant lineages identified in Oregon coast diatom bloom. Environ. Microbiol. **8:**1361–1370. http://dx.doi.org/10.1111/j.1462-2920.2006.01029.x.
12. **Glockner FO, Kube M, Bauer M, Teeling H, Lombardot T, Ludwig W, Gade D, Beck A, Borzym K, Heitmann K, Rabus R, Schlesner H, Amann R, Reinhardt R.** 2003. Complete genome sequence of the marine *Planctomycetes* Pirellula sp. strain 1. Proc. Natl. Acad. Sci. U. S. A. **100:**8298–8303. http://dx.doi.org/10.1073/pnas.1431443100.
13. **Woebken D, Teeling H, Wecker P, Dumitriu A, Kostadinov I, Delong EF, Amann R, Glöckner FO.** 2007. Fosmids of novel marine *Planctomycetes* from the Namibian and Oregon coast upwelling systems and their cross-comparison with planctomycete genomes. ISME J. **1:**419–435. http://dx.doi.org/10.1038/ismej.2007.63.

14. **Fuerst JA.** 1995. The *Planctomycetes*: emerging models for microbial ecology, evolution and cell biology. Microbiology **141:**1493–1506. http://dx.doi.org/10.1099/13500872-141-7-1493.
15. **Kirkpatrick J, Oakley B, Fuchsman C, Srinivasan S, Staley JT, Murray JW.** 2006. Diversity and distribution of *Planctomycetes* and related bacteria in the suboxic zone of the Black Sea. Appl. Environ. Microbiol. **72:**3079–3083. http://dx.doi.org/10.1128/AEM.72.4.3079-3083.2006.
16. **Gerdeaux D, Druart JC, Laine L, Lazzarotto J, Perga ME, Rimet F.** 2008. Suivi scientifique du lac d'Annecy. Rapport 2008. Syndicat Intercommunal du Lac d'Annecy, Annecy, France.
17. **Strickland JDH, Parsons TR.** 1968. A practical handbook of seawater analysis. Pigment analysis. Bull. Fish. Res. Board Canada **167:**1–311.
18. **Pernthaler A, Pernthaler J, Amann R.** 2002. Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. Appl. Environ. Microbiol. **68:**3094–3101. http://dx.doi.org/10.1128/AEM.68.6.3094-3101.2002.
19. **Dorigo U, Fontvieille D, Humbert JF.** 2006. Spatial variability in the abundance and composition of the free-living bacterioplankton community in the pelagic zone of Lake Bourget (France). FEMS Microbiol. Ecol. **58:**109–119. http://dx.doi.org/10.1111/j.1574-6941.2006.00139.x.
20. **Mühling M, Woolven-Allen J, Murrell JC, Jiant I.** 2008. Improved group specific PCR primers for denaturing gradient gel electrophoresis analysis of the genetic diversity of complex microbial communities. ISME J. **2:**379–392. http://dx.doi.org/10.1038/ismej.2007.97.
21. **Pollet T, Tadonléké DR, Humbert JF.** 2011. Comparison of primer sets for the study of *Planctomycetes* communities in lentic freshwater ecosystems. Environ. Microbiol. Rep. **3:**254–261. http://dx.doi.org/10.1111/j.1758-2229.2010.00219.x.
22. **Muyzer G, de Waal EC, Uitterlinden AG.** 1993. Profiling of complex microbial populations by denaturating gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. **59:**695–700.
23. **Schauer M, Balagué V, Pedrós-Alió C, Massana R.** 2003. Seasonal changes in the taxonomic composition of bacterioplankton in coastal oligotrophic system. Aquat. Microb. Ecol. **31:**163–174. http://dx.doi.org/10.3354/ame031163.
24. **Debroas D, Humbert JF, Enault F, Bronner G, Faubladier M, Cornillot E.** 2009. Metagenomic approach studying the taxonomic and functional diversity of the bacterial community in a mesotrophic lake (Lac du Bourget, France). Environ. Microbiol. **11:**2412–2424. http://dx.doi.org/10.1111/j.1462-2920.2009.01969.x.
25. **Humbert JF, Dorigo U, Cecchi P, LeBerre B, Debroas D, Bouvy M.** 2009. Comparison of the structure and composition of bacterial communities from temperate and tropical freshwater ecosystems. Environ. Microbiol. **11:**2339–2350. http://dx.doi.org/10.1111/j.1462-2920.2009.01960.x.
26. **Nicholas KB, Nicholas HBJ.** 1997. Genedoc: a tool for editing and annotating multiple sequence alignments. http://www.nrbsc.org/gfx/genedoc/.
27. **Huber T, Faulkner G, Hugenholtz P.** 2004. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. Bioinformatics **20:**2317–2319. http://dx.doi.org/10.1093/bioinformatics/bth226.
28. **Cole JR, Chai B, Farris RJ, Wang Q, Kulam SA, McGarrell DM, Marsh T, Garrity GM, Tiedje JM.** 2005. The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acid Res. **33**(Database issue):D294–D296. http://dx.doi.org/10.1093/nar/gki038.
29. **Kruskal JB, Wish M.** 1978. Multidimensional scaling. Sage University papers on quantitative applications in the social sciences 07011. Sage Publications, Beverly Hills, CA.
30. **Clarke KR.** 1993. Non-parametric multivariate analyses of changes in community structure. Aust. J. Ecol. **18:**117–143. http://dx.doi.org/10.1111/j.1442-9993.1993.tb00438.x.
31. **Clarke KR, Warwick RW.** 2001. Change in marine communities: an approach to statistical analysis and interpretation, 2nd ed. Primer-E, Plymouth, United Kingdom.
32. **Lozupone C, Knight R.** 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Appl. Environ. Microbiol. **71:**8228–8235. http://dx.doi.org/10.1128/AEM.71.12.8228-8235.2005.
33. **Fisher MM, Klug JL, Lauster G, Newton M, Triplett EW.** 2000. Effects of resources and trophic interactions on freshwater bacterioplankton diversity. Microb. Ecol. **40:**125–138. http://dx.doi.org/10.1007/s002480000049.
34. **Pinhassi J, Berman T.** 2003. Differential growth response of colony forming α- and γ-proteobacteria in dilution culture and nutrient addition experiments from Lake Kinneret (Israel), the eastern Mediterranean Sea, and the Gulf of Eilat. Appl. Environ. Microbiol. **69:**199–211. http://dx.doi.org/10.1128/AEM.69.1.199-211.2003.
35. **Jardillier L, Boucher D, Personnic S, Jacquet S, Thénot A, Sargos D, Amblard C, Debroas D.** 2005. Relative importance of nutrients and mortality factors on prokaryotic community composition in two lakes of different trophic status: microcosm experiments. FEMS Microbiol. Ecol. **53:**429–443. http://dx.doi.org/10.1016/j.femsec.2005.01.011.
36. **Šimek K, Horňák K, Jezbera J, Nedoma J, Vrba J, Straškrábová V, Macek M, Dolan JR, Hahn MW.** 2006. Maximum growth rates and possible life strategies of different bacterioplankton groups in relation to phosphorus availability in a freshwater reservoir. Environ. Microbiol. **8:**1613–1624. http://dx.doi.org/10.1111/j.1462-2920.2006.01053.x.
37. **Salcher MM, Hofer J, Horňák K, Jezbera J, Sonntag B, Vrba J, Šimek K, Posch T.** 2007. Modulation of microbial predator-prey dynamics by phosphorus availability: growth patterns and survival strategies of bacterial phylogenetic clades. FEMS Microbiol. Ecol. **60:**40–50. http://dx.doi.org/10.1111/j.1574-6941.2006.00274.x.
38. **Pradeep Ram AS, Sime-Ngando T.** 2008. Functional responses of prokaryotes and viruses to grazer effects and nutrient additions in freshwater microcosms. ISME J. **2:**498–509. http://dx.doi.org/10.1038/ismej.2008.15.
39. **Sakamoto M.** 1966. Primary production by the phytoplankton community in some Japanese lakes and its dependence on lake depth. Arch. Hydrobiol. **62:**1–28.
40. **Tadonléké RD, Lazzorotto J, Anneville O, Druart JC.** 2009. Phytoplankton productivity increased in Lake Geneva despite phosphorus loading reduction. J. Plankton Res. **31:**1179–1194. http://dx.doi.org/10.1093/plankt/fbp063.
41. **Azam F, Malfatti F.** 2007. Microbial structuring of marine ecosystems. Nat. Rev. Microbiol. **5:**782–791. http://dx.doi.org/10.1038/nrmicro1747.
42. **Akin N.** 2006. Effets des éléments nutritifs sur les communautés bactériennes planctoniques du lac d'Annecy. Rapport du Master 2. Université Claude Bernard, Lyon, France.
43. **Berdjeb L, Ghiglione JF, Jacquet S.** 2011. Bottom-up versus top-down control of hypo- and epilimnion free-living bacterial community structures in two neighboring freshwater lakes. Appl. Environ. Microbiol. **77:**3591–3599. http://dx.doi.org/10.1128/AEM.02739-10.
44. **Obernosterer I, Hendl GJ.** 1995. Phytoplankton extracellular release and bacterial growth: dependence on the inorganic N:P ratio. Mar. Ecol. Prog. Ser. **116:**247–257. http://dx.doi.org/10.3354/meps116247.
45. **Currie DJ, Kalff J.** 1984. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. Limnol. Oceanogr. **29:**298–310. http://dx.doi.org/10.4319/lo.1984.29.2.0298.
46. **Cotner JB, Hall EK, Scott JT, Heldal M.** 2010. Freshwater bacteria are stoichiometrically flexible with a nutrient composition similar to seston. Front. Microbiol. **1:**132. http://dx.doi.org/10.3389/fmicb.2010.00132.
47. **Nakamura K, Masuda K, Mikami E.** 1989. Polyphosphate accumulating bacteria and their ecological characteristics in activated sludge process, p 427–431. *In* Hattori T, Ishida Y, Maruyama Y, Morita RY, Uchida A (ed), Recent advances in microbial ecology. Japan Scientific Societies Press, Tokyo, Japan.
48. **Domaizon I, Viboud S, Fontvieille D.** 2003. Taxon-specific and seasonal variations in flagellates grazing on heterotrophic bacteria in the oligotrophic Lake Annecy: importance of mixotrophy. FEMS Microbiol. Ecol. **46:**317–329. http://dx.doi.org/10.1016/S0168-6496(03)00248-4.